Data Evaluation Record on the activated sludge respiration inhibition of cetyl pyridinium chloride (CPC monohydrate)

PMRA Submission Number {.....} EPA MRID Number 48012107

Data Requirement:	PMRA Data Code:	
	EPA DP Barcode:	376391
	OECD Data Point:	OECD 209
	EPA Guideline:	850.6800

Test material:

Common name: Cetyl pyridinium chloride.

Chemical name

IUPAC name: 1-Hexadecylpyridinium chloride.

CAS name: Not reported.

CAS No.: 6004-24-6 for monohydrate (123-03-5 for anhydrous).

Synonyms: Cetylpyridinium chloride.

Smiles string:

Primary Reviewer: James Breithaupt, Agronomist
Signature:
Antimicrobial Division (AD)
Date: 6/30/10

Risk Assessment and Science Support Branch (RASSB)

James Breithaupt
6/30/10

Final Reviewer: Nader ElkassabanyChief
Signature:
Antimicrobial Division (AD)
Date: 6/30/10

Risk Assessment and Science Support Branch (RASSB)

Company Code:
Active Code:
Use Site Category:
EPA PC Code: 069160

CITATION: Kelly, C.R., K. Paterson. 2005. CPC Monohydrate activated sludge, respiration inhibition test. Unpublished study performed by Inveresk, Tranent, Scotland, and sponsored and submitted by Rutherford Chemicals, Harriman, New York. Inveresk Report No.: 23065. Inveresk Study No. 804303. The experiment was initiated May 12, 2003 and completion May 29, 2003 (p. 10). Final report issued March 8, 2005.

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EXECUTIVE SUMMARY

Study Acceptability: This study is classified as acceptable and satisfies the OECD 209 data requirement. The reference compound 3,5-dichlorophenol demonstrated inhibition of about 7 %, which is between the acceptable 5-30 %. The endpoints were:

Maximum inhibition = 106.1% with a test substance concentration of 1000 mg/L.
EC_{50} = 20.7 mg/L (95% confidence interval of 19.8 – 21.7 mg/L).
EC_{20} = 10.4 mg/L (95% confidence interval of 9.7 – 11.1 mg/L).
EC_{80} = 41.2 mg/L (95% confidence interval of 39.0 – 43.7 mg/L).
NOEC = Not determined.

The effect of CPC monohydrate (cetyl pyridinium chloride; chemical purity 99.9%) on activated sludge respiration inhibition was studied in aerated activated sludge from Haddington, Scotland for 3 hours at 18-22°C. 3,5-Dichlorophenol was included as the reference substance. The study was conducted in accordance with OECD Guideline for Testing of Chemicals, No. 209, and in compliance with the USEPA, OECD Principles of Good Laboratory Practice. The activated sludge was collected from a municipal wastewater treatment plant which receives waste predominantly from domestic sources. After collection, the supernatant was decanted and on the day of use TSS was determined as 4.4 g/L. The activated sludge (200 mL) was mixed with synthetic sewage (16 mL), the appropriate amount of test and reference solution (in deionized water) were added, and brought to volume in a 500 mL test vessel with deionized water. Samples were aerated using compressed air (zero grade, BOC gases) at a flow rate of 0.6-0.8 L/min. The air flow to each vessel was regulated using an air trap and an air stone used to increase aeration.

The nominal test concentrations of CPC monohydrate were 10, 31.6, 100, 316 and 1000 mg/L. The nominal concentrations of the reference substance, 3,5-dichlorophenol, were 5, 15 and 30 mg/L. Two controls were included in the experiment; these controls contained only synthetic sewage feed and deionized water. The incubation of the samples was separated by 15-minute intervals; the first and last samples of the sample set were the controls. After 3 hours of incubation, the contents each vessel was poured to overflowing into a glass Biological Oxygen Demand (BOD) bottle (250 mL) and the dissolved oxygen concentration measured at 30 second intervals for up to 10 minutes, or until the last reading was <2.5 O_2/L was observed.

The temperature ranged from 18.37-20.67°C throughout the study period; the pH at the start of contact time ranged from 6.9-7.3 and at the end of contact time was 7.1-7.9. The dissolved oxygen (DO) content after 3 hours of contact time with the test substance at the 10 mg/L test concentration was 7.07 mg/L at 0.5 min. and 2.25 mg/L at 6.5 min.; at the 31.6 mg/L test concentration DO was 8.03 mg/L at 0.5 min. and 5.21 mg/L at 10.0 min.; at the 100 mg/L test concentration DO was 8.49 mg/L at 0.5 min. and 8.22 at 10.0 min.; at the 316 mg/L test concentration DO was 8.69 mg/L at 0.5 min. and 8.72 mg/L at 10.0 min.; and at the 1000 mg/L test concentration DO was 9.11 mg/L at 0.5 min. and 9.26 mg/L at 3.0 min.

For CPC monohydrate (cetyl pyridinium chloride), the maximum inhibition was 106.1% with a test substance nominal concentration of 1000 mg/L. At 10, 31.6, 100, and 316 mg/L CPC monohydrate produced inhibition rates of 18.6%, 70.0%, 97.1%, and 100.3%, respectively. The EC_{50} value was 20.7 mg/L (95% confidence interval of 19.8-21.7%). The 3 hour EC_{20} and EC_{80} for CPC monohydrate was 10.4 and 41.2 mg/L, respectively (95% confidence intervals of 9.7-11.1 mg/L and 39.0-43.7 mg/L, respectively). For 3,5-dichlorophenol, the EC_{50} value was 7.4 mg/L (95% confidence interval not calculated).

At the 1000 mg/L test concentration, the solution frothed excessively during the 3 hour contact time and during the respiration rate investigation, readings were stopped after 3 minutes due to increasing dissolved oxygen concentrations. At the end of the 3 hour contact time, the 316 and 1000 mg/L test concentrations were clear and almost colorless, indicating mortality of the sludge inoculum over the 3 hour exposure period at these two highest test concentrations.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study was conducted in accordance with OECD Guideline 209 and Council of European Communities Guideline C11 (p. 10). No significant deviation from the objectives of OECD No. 209 was noted.

COMPLIANCE: This study was conducted in compliance with the USEPA GLP (40 CFR, Part 160; 1989), and UK GLP Directive 2004/9/EC (pp. 3-4). Signed and dated Data Confidentiality, GLP, Quality Assurance and Authentication statements were provided (pp. 2-4, 6-7).

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A. MATERIALS:

1. Test Material CPC Monohydrate (cetylpyridinium chloride; p. 9).

Chemical Structure:

Description: Off-white powder (p. 11).

Purity:

Radiochemical purity:	Not applicable.
Lot/Batch No.:	00217109.
Analytical purity:	99.9% (Expiration date October 6, 2007).
Specific activity:	Not applicable.
Location of the radiolabel:	Not radiolabeled.

Storage conditions of test chemical: Ambient conditions in darkness.

2. Reference Material 3,5-Dichlorophenol (3,5-DCP; p. 11).

Chemical Structure:

Description: Solid.

Purity:

Radiochemical purity:	Not applicable.
Lot/Batch No.:	80703 ES02611ES.
Analytical purity:	97.0%.
Specific activity:	Not applicable.
Location of the radiolabel:	Not radiolabeled.

Storage conditions of test chemical: The test substance was stored at ambient conditions in the dark (p. 11).

Physico-chemical properties of CPC monohydrate:

Parameter	Value	Comment
Molecular formula	Not reported.	
Molecular weight	Not reported.	
Water solubility	Not reported.	
Vapor pressure/Volatility	Not reported.	
UV Absorption	Not reported.	
Dissociation constant (pKa)	Not reported.	
Partition coefficient (octanol/water) log K_{ow}/K_{ow}	Not reported.	
Stability of compound at room temperature	Not reported.	

3. Activated Sludge Characteristics

Table 1: Description of activated sludge.

Description	Details
Geographic location	Haddington Municipal Sewage Works, a local (Inveresk, Scotland) sewage processing plant handling predominately domestic sewage.
Pesticide use history at the collection site	Not reported.
Collection date	May 27, 2003.
Collection procedures	The overlying supernatant was siphoned off and retained, while the remaining sludge was shaken by hand to homogenize.
Sampling depth	Not reported.
Storage conditions	Not reported.
Storage length	None before processing; 1 day, overnight, after processing.

Data obtained from p. 11 of the study report.

Preparation/Processing: Sub-samples (5 mL) of the homogenized sludge was oven-dried (*ca.* 105°C) and the total suspended solids (TSS) determined as 5.2 g/L on the day of collection (p. 11). The sludge was mixed daily with 50 mL/L of synthetic sewage and aerated vigorously at 18-22°C prior to use (Appendix 2, p. 22). On the day of use (May 29, 2003), the TSS was re-determined as 4.4 g/L. The sludge was retained for use for more than 24 hours; however, the validity test for sensitivity of the inoculum was acceptable therefore this deviation did not affect the study outcome.

4. Synthetic sewage feed preparation

The synthetic sewage feed was prepared by dissolving the following substances in 500 mL of deionised water: 8.0 g peptone; 5.5 g meat extract; 1.5 g urea; 0.35 g NaCl; 0.2 g $CaCl_2$ x $2H_2O$; 0.1 g $MgSO_4$ x $7H_2O$; and 1.4 g K_2HPO_4 (p. 11; Appendix 2, p. 22). Each batch was used within 24 hours.

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: None reported.

2. Experimental conditions:

Table 2: Study design.

Parameter		**Test substance**	**Reference substance**
Duration of the test		3 hours of incubation at each concentration.	
Activated sludge condition	Preparation	16 mL of synthetic sewage feed was brought up to 300 mL with deionised water.	
	pH	Initial: 6.9-7.3. Final: 7.1-7.9.	
Mixed liquor suspended solids level (mg/L)		4400 mg/L.	
Amount of activated sludge per treatment		200 mL (final volume 500 mL).	
Test materials	Stock solutions preparation	CPC monohydrate (1.25 g) was added to 250 mL deionized water and ultrasonicated for ca. 10 minutes. The final concentration of CPC monohydrate was 5 g/L.	3,5-dichlorophenol (0.1003 g) was dissolved in 100 mL deionized water and ultrasonicated for *ca.* 20 minutes. The Final concentration of 3,6-DCP was 1 g/L.
	Test concentrations (nominal)	10, 31.6, 100, 316, and 1000 mg/L.	5, 15 and 30 mg/L.
Control conditions, if used		The same as those of the test and reference samples.	
No. of Replications	Controls, if used	Two samples total for study.	
	Treatment	One sample per test concentration was prepared.	
Test apparatus	Type/material/volume	Each 500-mL test vessel (not described) was prepared with 16 mL synthetic sewage feed, 200 mL activated sludge and the appropriate amount of the test substance or reference substance stock solution, then diluted to 500 mL with deionized water.	
	Details of traps for CO_2 and organic volatiles, if any	Traps not used. Each vessel was capped with a foam bung.	
If no traps were used, is the system closed/open?		Not applicable.	
Identity and concentration of co-solvent		None.	
Test material	Volume of the test solution used/treatment:	1, 3.16, 10, 31.6 and 100 mL.	2.5, 7.5, and 15 mL.
	Application method :	Not reported.	
	Is the co-solvent evaporated?	Not applicable.	
Any indication of the test material adsorbing to the walls of the test apparatus?		None.	
Microbial biomass of the control (mg microbial carbon)		Not determined.	
Microbial biomass (mg microbial carbon)		Not determined.	
Experimental conditions:	Temperature (°C):	18-22°C water bath.	
	Continuous darkness:	Not applicable.	
	Aeration:	Aerated using compressed air (zero grade, BOC gases) at a ~~flow rate of 0.6-0.8 L/min. The air flow to each vessel was~~ regulated using an air trap and an air stone used to increase	

Parameter		Test substance	Reference substance
		aeration.	
	Intervals between samples:	Each sample concentration was prepared at 15 minutes intervals.	
Other details, if any		The incubation of the samples was separated by 15-minute intervals; the first and last samples of the sample set were the controls.	

Data were obtained from pp. 12-13; Table 2, p. 18 of the study report.

3. Aerobic/anaerobic conditions: Samples were aerated using compressed air (zero grade, BOC gases) at a flow rate of 0.6-0.8 L/min (pp. 12-13). The air flow to each vessel was regulated using an air trap and an air stone used to increase aeration. After 3 hours of incubation, the contents of the control vessel (C1) was poured to overflowing into a glass Biological Oxygen Demand bottle (250 mL) and the dissolved oxygen concentration measured at 30 second intervals for up to 10 minutes, or until the last reading was <2.5 O_2/L was observed (p. 13; Appendix 3, p. 23).

4. Supplementary experiments: None reported.

5. Sampling:

Table 3: Sampling details.

Criteria	Details
Sampling intervals	3 hours of incubation.
Sampling method	At the end of incubation, the content of each vessel was poured to overflowing into a glass Biological Oxygen Demand (BOD) bottle (250 mL).
Method of collection of CO_2 and organic volatile compounds	Volatiles were not collected.
Sampling intervals/times for:	
Sterility check, if sterile controls are used:	Not applicable.
Moisture content:	Not applicable.
pH levels:	Measured at start and end time.
Redox potential:	Not applicable.
Dissolved oxygen:	The dissolved oxygen concentration in the BOD bottle was measured at 30 second intervals for up to 10 minutes, or until the last reading was <2.5 O_2/L was observed
Sample storage before analysis	None.
Other observation, if any	None.

Data were obtained from p. 13, Table 2, p. 18 of the study report.

C. ANALYTICAL METHODS:

Statistical Analysis: A probit transformation was applied to the respiration inhibition values, expressed as a percentage of the mean control value, and subjected to regression against log

transformed concentrations where appropriate (p. 13). The Davidon-Fletcher-Powell maximum likelihood algorithm was used to obtain parameter estimates and the EC_{50} estimated from the fitted model.

For the reference data, the Pearson Chi-square Test on the sum of squares was used to indicated heterogeneity was large ($p<0.05$), and effect of a limited data set (3 concentrations) and limited intermediate responses (p. 13). However, the EC_{50} estimate from graphical representation was regarded as acceptable. The EC_{20} and EC_{90} value and 95% confidence intervals were not reported.

II. RESULTS AND DISCUSSION

A. TEST CONDITIONS: The temperature ranged from 18.37-20.67°C throughout the study period (Table 2, p. 18). The pH at the start of contact time ranged from 6.9-7.3 and at the end of contact time was 7.1-7.9. The dissolved oxygen (DO) content after 3 hours of contact time with the test substance at the 10 mg/L test concentration was 7.07 mg/L at 0.5 min. and 2.25 mg/L at 6.5 min.; at the 31.6 mg/L test concentration DO was 8.03 mg/L at 0.5 min. and 5.21 mg/L at 10.0 min.; at the 100 mg/L test concentration DO was 8.49 mg/L at 0.5 min. and 8.22 at 10.0 min.; at the 316 mg/L test concentration DO was 8.69 mg/L at 0.5 min. and 8.72 mg/L at 10.0 min.; and at the 1000 mg/L test concentration DO was 9.11 mg/L at 0.5 min. and 9.26 mg/L at 3.0 min. (Appendix 3, p. 23).

Table 4: Respiration rates and percent inhibition results of the activated sludge respiration inhibition test with CPC monohydrate (cetyl pyridinium chloride) and the reference substance (3,5-dichlorophenol).

Test sample	Nominal concentration of substance (mg/L)	Respiration rate (mg O_2 L^{-1} h^{-1})	% inhibition
CPC Monohydrate	10	48.00	18.61
	31.6	17.70	69.99
	100	1.71	97.10
	316	-0.19	100.32
	1000	-3.60	106.10*
3,5-Dichlorophenol	5	38.18	35.26
	15	14.10	76.09
	30	7.45	87.37
Control 1	0.0	61.89	NA
Control 2	0.0	56.06	NA
Mean of Controls	0.0	58.98	NA
SD	0.0	4.12	NA

Data were obtained from Table 1, p. 17 of the study report.
*Not used in probit analysis due to excessive frothing.
NA = Not applicable.

B. RESULTS: For CPC monohydrate (cetyl pyridinium chloride), the maximum inhibition was 106.1% with a test substance nominal concentration of 1000 mg/L (pp. 14-15; Table 1, p. 17). At 10, 31.6, 100, and 316 mg/L CPC monohydrate produced inhibition rates of 18.6%, 70.0%, 97.1%, and 100.3%, respectively. The EC_{50} value was 20.7 mg/L (95% confidence interval of 19.8-21.7%). The 3 hour EC_{20} and EC_{80} for CPC monohydrate was 10.4 and 41.2 mg/L, respectively (95% confidence intervals of 9.7-11.1 mg/L and 39.0-43.7 mg/L, respectively). For 3,5-dichlorophenol, the EC_{50} value was 7.4 mg/L (95% confidence interval not calculated).

The study authors included graphical representations of the percent inhibition versus concentration for CPC monohydrate and 3,5-dichlorophenol (Figures 1-2, pp. 19-20).

At the 1000 mg/L test concentration, the solution frothed excessively during the 3 hour contact time and during the respiration rate investigation, readings were stopped after 3 minutes due to increasing dissolved oxygen concentrations (p. 14). At the end of the 3 hour contact time, the 316 and 1000 mg/L test concentrations were clear and almost colorless, indicating mortality of the sludge inoculum over the 3 hour exposure period at these two highest test concentrations.

C. SUPPLEMENTARY EXPERIMENT-RESULTS: No supplemental experiments were reported.

III. STUDY DEFICIENCIES

No significant deviations from OECD Guideline No. 209 were noted.

IV. REVIEWER'S COMMENTS

1. The program used for conducting probit analysis was validated at Inveresk (Inveresk Report No. 21952) and determined suitable for use (p. 13).

2. The two points of validity criteria of OECD Guideline No. 209 were satisfied by the study (p. 14). The respiration rates of the two controls were 56.06 and 58.98 mg O_2 L^{-1} h^{-1}, respectively, which was a difference of 9.4% after 3 hour contact time (Table 1, p. 17). The validity criterion of the OECD Guideline No. 209 is that the difference between the respiration rates of the two controls is 15% or less. The experimentally determined EC_{50} value of the reference substance 3,5-dichlorophenol was 7.4 mg/L after 3 hours of contact time; the range prescribed by the OECD Guideline No. 209 is 5 to 30 mg/L. Finally, the lowest dissolved oxygen level recorded at the start of the respiration rate phase was 7.04 mg/L indicating sufficient aeration during the 3 hour contact time; OECD guidance criterion is >6.5 mg/L.

V. REFERENCES

1. OECD Guideline for Testing of Chemicals, No. 209, Activated Sludge, Respiration Inhibition Test. 1984.

2. U.S. Environmental Protection Agency. 1996. Ecological Effects Test Guidelines, No. 850.6800, Modified Activated Sludge, respiration Inhibition Test for Sparingly Soluble Chemicals. Office of Prevention, Pesticides and Toxic Substances, Washington, DC. EPA 712-C-96-168.

3. U.S. Environmental Protection Agency. 1989. FIFRA Accelerated Reregistration, Phase 3 Technical Guidance. Office of Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 540/09-90-078.

4. U.S. Environmental Protection Agency. 1993. Pesticide Registration Rejection Rate Analysis - Environmental Fate. Office of Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 738-R-93-010.